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Refinery of Biomass by Utilization of Specific Effects of Microwave Irradiation

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Abstract

The results of application of microwave (MW) irradiation to achieve refinery of various kinds of recalcitrant biomass were summarized with special emphasis on recent innovative utilization of sensitizers for MW irradiation. The saccharification rates of lignocellulosic plant biomass consisting of softwoods, hardwoods and monocotyledons attained by the simple MW irradiation treatment were in the orders of 35-65% (softwoods), 70-80% (hardwoods) and around 80% (monocotyledons), respectively. The corresponding values of cellulose were around 69-81% (230-240 °C), while xylan was susceptible to the MW energy and easily decomposed into oligomers including xylose by heating at >150 °C, optimally at 204 °C, to give 99% solubility rate. However, the effects of the MW treatment on the agro-food byproducts varied highly depending upon their origins. These results indicate necessity of troublesome specification of the optimum condition for each case. For development of more versatile and comprehensive refinery method we tried to use sensitizers for MW irradiation.We found that hydrogen peroxide was remarkably effective for biomass refinery. Utilization of activated carbon as a sensitizer for MW irradiation was also effective for saccharification of starchy materials. Enhancement of the surface charge of the activated carbons by oxidation and preparation of microwave absorption solid acid catalysts were recommended to further enhance their effectiveness. The overall results showed the importance of four factors, less affinity toward oligosaccharides in relation with pore size, quantity of surface negative charges in addition to electro-conductivity, faster filtration ability and unknown hot spot productivity. Due to removal of the coloured materials by the activated carbons, clear saccharified solution produced by one pot conversion could be directly used for further fermentation to produce biofuels such as ethanol.

Keywords: Microwave; refinery; biomass; hydrogen peroxide; activated carbon.

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1. Introduction

Utilization of land-plant biomass as a source of organic compounds which are available from fossil resources is one of goals for us to maintain sustainable development of humanosphere. However diversity and recalcitrance of the land-plants hamper their practical use. The techniques for overcoming the recalcitrance are usually called as pretreatments. So far a wide variety of techniques have been developed to overcome the recalcitrance, e.g. highly crystalline state of cellulose, for enhancement of enzymatic digestibility of cellulose [1]. Our aim is to utilize microwave irradiation as a kind of the pretreatment technologies for utilization of the land-plant biomass as valuable resources.

Microwave (MW) irradiation is a kind of green technology using dielectric heating of the materials by dielectric loss due to dipolar relaxation of the dipoles in the solvent and conduction loss of the solid materials. This technique has many advantages such as a shorter time, less or without solvent, higher extraction rate, homogeneous heating with internal heating. Our application of MW irradiation for utilization of recalcitrant biomass has been carried out as a pioneer since 1984 [2].The whole results [3] will be summarized in this study with special emphasis on recent innovative applications by using sensitizers which enhance the effects of the MW irradiation on biomass [4-7].

Firstly hydrogen peroxide was used as a sensitizer [4]. By application of the MW energy to woody biomass in the presence of ≤10 % H_2O_2, its three major components, lignin, hemicellulose and cellulose, became separable by lower temperature treatment at 160 °C (0.6 MPa) for ≤7 min than usual hydrothermal heating at >220 °C (>2.3 MPa) accompanied by appreciable depolymerization of hemicellulose and lignin.

Secondary activated carbon which can act as hot spots in the MW environment was used as a sensitizer for degradation of starch [5-7]. The results clearly showed that carbons having low adsorbing capacity of oligosaccharides accelerated the conversion into glucose accompanied by decrease in the optimal heating temperature by 10-30 °C with adsorption of coloured materials on the carbons. In addition, carbons with higher affinity to oligomers were usable for production of oligomers which could be recovered by elution with aqueous ethanol [7]. An extended research work in this line of investigation is also included in this study by using sulfuric acid on activated carbon as a new microwave absorption solid state catalyst.

In this study, the authors lastly introduced development of a continuous flow type MW system and present its utilization for saccharification of starchy biomass.

2. Experiment

2.1. Materials

All land-plant biomass samples were the same as those cited in the references [3,7]. Six kinds of activated carbons (A-F), graphite, single-walled (SW) and multi-walled (MW) carbon nanotubes (CNTs) were the same as described previously [5]. The adsorption capacities of these activated carbons for maltose are 145.8 (A, powder), 21.5 (B, fractured), 23.2 (C, fractured), 28.6 (D, fractured), 134.0 (E, fractured) and 48.3 (F, granule) mg/g, respectively. In addition, MW-CNTs having 50-100 nm, length 5-15 μm and EC ≧10 S/cm (MW-CNT1) and 8-15 nm, length ± 30μm and EC ≧102 S/cm (MW-CNT2), and a MW-CNT3 functionalized with COOH by treatment of MW-CNT2 with a 1:3 (v/v) mixture of 97% sulfuric acid and 65% nitric acid for 6 h at 120 °C were also used as sensitizers for MW irradiation.

Microwave absorption solid acid catalysts, sulfuric acid on activated carbons (H_2SO_4/C), were prepared by a slight modification of the method of Yuan *et al.* [8]. Briefly prewashed and dried 10 g of the activated carbon B was impregnated with 50 g each of 36, 50, 65 and 72 wt% sulfuric acid at room temperature for 12 h. Water was removed by rotary evaporator at 80 ^oC and heating in the furnace for 1 h

at 200 °C. After cooling to room temperature, each solid catalyst was washed with pure water until pH of the washed water became constant and dried at 105 °C.

2.2. Microwave irradiation treatment

Basically suspension of each sample in water (1 g/20 mL or 5% w/v) with the carbon material (1 g) was prepared in a 100 mL Teflon® tube and subjected to MW irradiation using MycroSYNTH Lab Station (≤1 kW, 2.45 GHz) microwave oven (Figure 1; Milestone Inc., Shelton, CT, USA) at temperature of 180-230 °C with pre-heating time of 4 minutes and heating time of 5-14 minutes. The samples were cooled immediately in an ice bath. Saccharification rate (%) was estimated as glucose yield as described previously [2,5]. A continuous flow type MW irradiation system originally constructed in 1988-1994 [9,10] was improved to have a capacity of 30 kg/h at 5.0 kW (2.45 GHz) equipped with two magnetrons (Japan Chemical Engineering & Machinery, Co., Ltd., http://www.nikkaki.co.jp/) as shown in Figure 2.

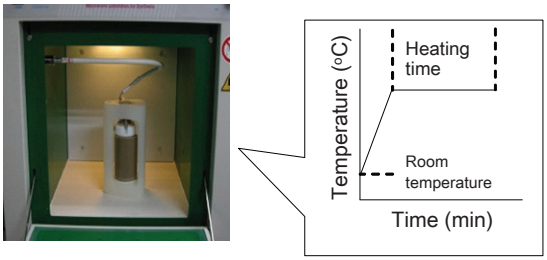

Fig. 1. Batch-type MW irradiation equipment

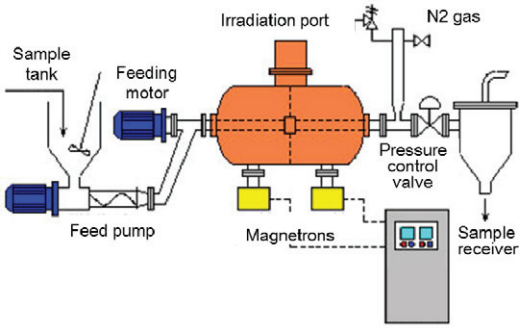

Fig. 2. Continuous flow MW irradiation system

3. Results and Discussion

3.1. Simple microwave irradiation of land-plant meals

Firstly, effects of the simple MW irradiation on land-plant meals were analyzed by grouping the samples into four types, softwoods, hardwoods, monocotyledons and agro-food by-products. The results were summarized in Figures 3 and 4. The results indicate easiness of degradation of hardwoods (70-80%) in comparison with softwoods whose saccharification rates were rather low in a range of 35-65%. Around 80% saccharification rates were also observed in the cases of the monocotyledonous biomass (Figure 3). However, the effects of the MW treatment on the agro-food residues changed highly depending upon species (Figure 4, including a result of marine algae (*Laminaria*)). Effects of the simple MW irradiation on components of the land-plant biomass were also analyzed [3,11]. Degradation of cellulose occurred by MW irradiation at ≧230 °C, and its saccharification rate attained 69-81% (230 - 240 °C). Xylan and arabinoxylan, the major hemicellulose in the hardwoods and monocotyledons, were susceptible to the MW energy and easily decomposed into oligomers including xylose by heating at > 150 °C, optimally at 204 °C, to give 99% solubilization rate. Lignin molecules present in hardwoods and monocotyledons were also susceptible to the MW energy at ≧180-190 °C and decomposed into lower molecular weight fragments of 3,400-15,000 which are soluble in aqueous acetone, methanol and 1,4-dioxane.

However softwood lignin is easy to condensate and precipitated during MW irradiation leading to wrapping the remained materials which hampered enzymatic attack or further degradation. Therefore MW assisted solvolysis in phenolic compounds or even alcohols with a few % of sulfuric acid as a catalyst are effective [1]. Recently use of green solvents such as ionic liquids has gained importance [1].

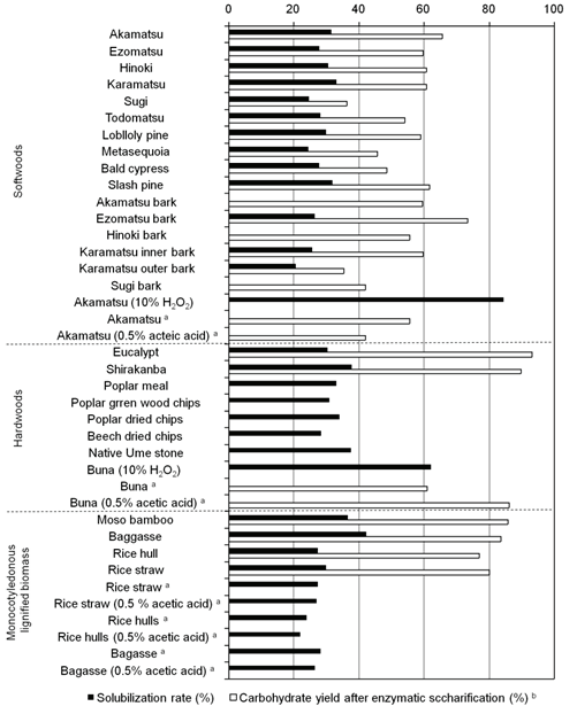

Fig. 3. Solubilization and saccharification rates (%) of three types of land-plant biomass (softwood, hardwood and monocotyledonous plant biomass). [a]Values obtained by using continuous MW irradiation system. [b]Values on the basis of carbohydrates. (cited from Tsubaki et al. [3])

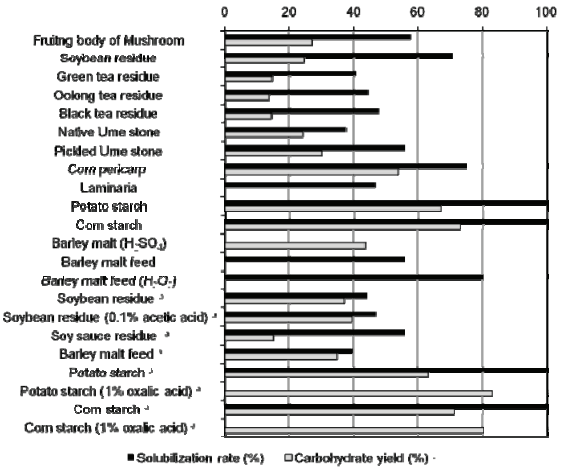

Fig. 4. Solubilization and saccharification rates (%) of agro-food by-products. [a,b]See in Fig. 3. (cited from Tsubaki et al. [3])

In addition, MW irradiation in the acidic conditions (acid hydrolysis pretreatment) is also known to be very much effective for saccharification of lignocellulosic land-plant biomass. Usually, acid pretreatment was carried out under diluted acids (0.2-2.5%) or concentrated acids. The dilute acid pretreatments are effective for selective degradation of hemicellulose, especially xylan, to monomers. This process has a merit not to use costly enzymes for production of fermentable sugars [1,3,11], but could not degrade crystalline cellulose. Treatment with concentrated mineral acids such as sulfuric acid is effective to solubilize and decompose crystalline cellulose, but necessitates two-step hydrolysis for production of monomeric sugars.

The values of solubilization and saccharification rates for the land-plant biomass attained by the MW treatment varied depending upon types and species. This indicates that troublesome determination of optimizing irradiation conditions for each biomass is indispensable to obtain superior results. Therefore development of more versatile and comprehensive methods is desired ardently in recent years.

3.2. *Utilization of hydrogen peroxide as a sensitizer of microwave irradiation*

During application of the MW irradiation technique to heat up biomass we happened to find out that hydrogen peroxide is usable for solubilization of biomass irrespective to types and species. Hydrogen peroxide is toxic but environmental friendly because water is its final product. In this paper we present usability of MW irradiation in the presence of hydrogen peroxide for refinery of three groups of land-plant biomass, softwood (Akamatsu, *pinus densiflora* Sieb. et Zucc.), hardwood (Beech, *Fagus crenata* Blume), and monocotyledon (Moso-bamboo, *Phyllostachys pubscens* Mazel) [4].

Table 1. Neutral sugar composition of the residues remained after MW irradiation of Akamatsu

Temp. (°C)	Ara	Gal	Glc	Xyl	Man
120	2.5	2.7	45.5	18.1	26.8
130	1.7	2.0	54.7	17.3	24.1
140	0.8	1.3	62.1	16.7	19.1
150	0.4	0.8	87.3	4.1	7.4
160	0.3	0	97.3	2.2	0

Concentration dependence of solubility of the sapwood of Akamatsu is firstly checked. The results shown in Figure 5 indicate the concentration dependent decrease in lignin content remained in the residue and a plateau at ≧5%. Therefore we used 10% hydrogen peroxide for the refinery (Figure 6). The highest solubility and extraction rates of lignin attained at 84.4% and 68.4% for Akamatsu, 62.3% and 91.8% for Beech and 56.7% and 47.6% for Moso-bamboo, respectively. Neutral sugar composition shown in Table 1 also shows remaining of cellulose at the final stage. Molecular weight distribution of the lignin solubilized by 90% aqueous acetone listed in the Table 2 indicates occurrence of fragmentation into molecular mass range (3-6 kDa) lower than milled wood lignin (8-10 kDa).

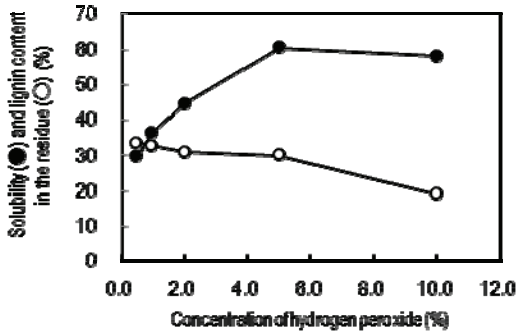

Fig. 5. Effects of hydrogen peroxide on solubility

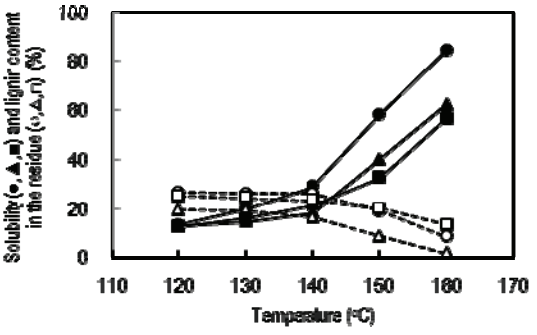

Fig. 6. Temperature dependence of solubility (10% H_2O_2)

(Heating temperature was set at 160 °C) (●, ○: Akamatsu; ▲,△: Beech; ■, □: Moso-bamboo)

As a result, three major polymers in woody materials, lignin, hemicellulose and cellulose, became separable by appreciably lower temperature treatment at 160 °C (0.6 MPa) by MW irradiation in the presence of hydrogen peroxide in comparison with conventional hydrothermal heating condition at >220 °C (>2.3 MPa). Although hydrogen peroxide is stable under acidic condition like in the present media, the present results showed usability of this reagent to assist the biomass refinery.

Table 2. Molecular mass of lignin (kDa) solubilized from Akamatsu by MW irradiation

Temp. (°C)	Akamatsu	Beech	Moso-bamboo
120	<2.0	<2.0	4.6
130	4.0	3.5	5.4
140	4.8	4.5	5.8
150	6.0	5.0	7.6
160	6.0	5.0	5.8

3.3. Utilization of activated carbon as a sensitizer of microwave irradiation

Use of solid state MW sensitizer is desirable for refinery of biomass because of easiness of removal after use. We looked for candidates for this purpose, and finally found that carbon materials fit for our line of investigation. The carbon materials absorb MW energy due to conduction loss effects, and are known to heat up to 1,283 °C in 1 minute by MW treatment of 25 g in a 1 L of vented water load in 1 kW MW oven (2.45 GHz) [12]. Sometimes catalytic reaction due to hot spots or so called microplasmas occurred on their surfaces [13].We therefore analyzed effects of activated carbon on oligosaccharides and starchy materials in water.

We first analyzed effects of activated carbon A on oligosaccahrides [7]. When an aqueous maltose solution (250 mg/20 mL) was heated by MW irradiation in water at 220-240 °C for 10 min, all materials were converted to glucose. Addition of this activated carbon in the reaction media, however, greatly suppressed formation of glucose to a range of 57.9-0.4% at 220 °C in good co-relationship with increasing amount of the activated carbon applied (up to 2 g). Analysis of the adsorbed materials after desorption with 50% aqueous ethanol indicated recovery of intact maltose at 93% at 220 °C. Similar observation was observed in the cases of the other oligosaccharides (cellobiose, sucrose and lactose) when applied with the same activated carbon.

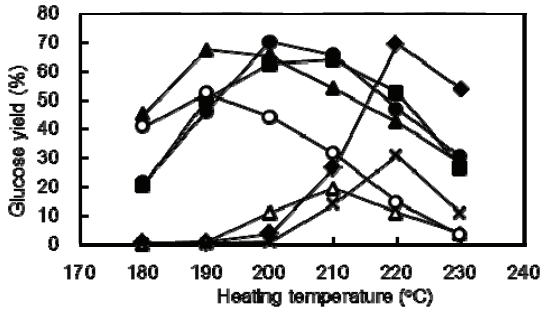

Fig. 7. Effects of activated carbons on glucose yields obtained by MW irradiation of corn starch at 1 g/20 mL for 12 min with heating up time of 4 min. Glucose yields obtained without activated carbon (♦), with activated carbons of A (Δ), B (●), C (▲), D (■), E (×), F (○) and without activated carbon (♦)

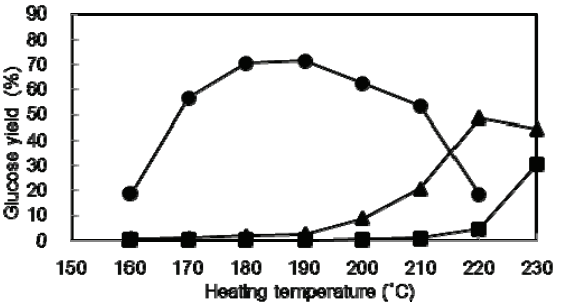

Fig. 8. Effects of MW absorption carbon materials on saccharification of cassava pulp. (■: Without carbon; ▲: with carbon B; ●: with 50% H_2SO_4/C)

We second intended to analyze the effects of activated carbons on saccharification of corn starch in water under MW electromagnetic field by using six types of activated carbons (A-F).The astonished results we have got are that the activated carbons with low adsorptive capacity of malto-oligosaccharides such as B, C and D, showed high saccharification rates, while those with high adsorptive capacity such as

A and E, exhibited low saccharification rates [5], Figure 7. The activated carbon F which have a moderate property showed to have a moderate rate of saccharification. In addition, the activated carbons B and C decreased the temperature necessary for saccharification by 10-30 °C. These results strongly support the contribution of the hot spots formed depending on the surface architecture of the activated carbons. For practical use filtration ability of the activated carbons was also very important to recover saccharified solution quickly after treatment. Based on this criterion the fractured or granule type activated carbons were adequate for saccharification of starchy polysaccharides. In addition, HPLC analysis of the materials recovered from the activated carbon A indicated that 20.8% of the materials applied could be recovered as a mixture of malto-oligosaccharides comprising from maltose to at least maltononaose. These results confirmed that oligosaccharides adsorbed on activated carbon showed great tolerance against breaking down into constituent monosaccharides by MW irradiation. The present results, however, in turn opened a new field of utilization of activated carbon for production of oligosaccharides from polysaccharides by using the MW irradiation technique.

Table 3. Effects of carbon materials on glucose yields obtained by MW irradiation of cassava pulp at 1 g/20 mL for 12 min with heating up time of 4 min.

Carbon	Glucose yield (%)	Carbon	Glucose yield (%)
Carbon B	48.9	Without carbon B	30.7
Graphite	41.5	36% H_2SO_4/C	70.8
SW-CNT	30.5	50% H_2SO_4/C	71.2
MW-CNT1	23.1	65% H_2SO_4/C	71.0
MW-CNT2	35.9	72% H_2SO_4/C	70.4
MW-CNT3	36.4		

However, only a limited positive effect was given on account of the other carbon materials such as graphite, SW-CNT and MW-CNT(Sigma); glucose yields from corn starch being 56.3% (graphite), 51.7% (SW-CNT) and 53.0% (MW-CNT, Sigma) at 220 °C, respectively [5]. Since these carbon materials do not have any porous structure, porous structure is suggested to be indispensable for the carbon materials to show sensitizing effects. Recently the effectiveness of the addition of the activated carbon C during the MW irradiation of cassava pulp was further shown by Hermiati *et al.* [6]. In these cases, glucose yields improved from 32.4% at 230 °C without using the activated carbon to 52.3% at 210 °C by MW irradiation for 12 min at 1 g activated carbon/1 g cassava pulp in 20 mL of water with suppression of the colouring materials. This method provides one pot glucose production method from starchy materials allowing simultaneous gelatinization, liquefaction, saccharification and removal of fermentation inhibitors. The effects of CNTs on glucose yield from cassava pulp were further analysed to get new information of the activation mechanisms induced by carbon by using MW-CNTs which had different electro-conductivity and surface charge. The results included in Table 3 not only clearly confirmed their low accessibilities as described above for corn starch [5], but also showed that MW-CNT3 which has higher electro-conductivity with COOH groups produced the highest glucose among the MW-CNTs, confirming further the importance of electro-conductivity and charge on their surfaces to show sensitizing effects. Use of activated carbon as a sensitizing agent during the MW irradiation was further extended to prepare the MW absorption solid acid catalysts, namely sulfuric acid on activated carbons (H_2SO_4/C). The original method of Yuan *et al.* [8] was successfully modified to prepare dried H_2SO_4/C catalysts treated with 36, 50, 65 and 72% sulfuric acid. When the prepared solid acid catalysts were used to saccharify the cassava pulp, the optimum heating temperature for glucose production could be lowered from 210 °C to around 180 °C with higher glucose yield as shown in Figure 8. The concentration of sulfuric acid for fitting the optimum glucose yield was also determined to be 50% by

comparing the glucose yields of four catalysts (Table 3). The all solutions containing saccharified glucose were colorless when the activated carbon B was used as a sensitizer.

The overall results on activities of the activated carbons on saccharification of starchy materials may be suffice to show the importance of four factors, less affinity toward oligosaccharides in relation with pore size, quantity of surface negative charges in addition to electro-conductivity, faster filtration ability and unknown hot spot productivity.

3.4. Utilization of continuous flow type MW irradiation system for saccharification of starch

The results obtained by the batch type equipment were successfully scaled up by using the continuous flow system, Figure 2 at 5-50% (w/w) and 2.4-20.0 L/h for various types of land plants, Figure 3 and agro-food by products, Figure 4 [3]. As a typical case glucose yield from corn starch, 71.0% at 220 °C, is comparable to 69.4% at 220 °C [6] given by the batch type MW irradiation.

4. Conclusion

Addition of hydrogen peroxide in water for MW irradiation was concluded to be remarkably effective for refinery of biomass and utilization of activated carbons as a sensitizer was also effective for saccharification of starchy materials. Enhancement of the surface charge of the activated carbon by oxidation and preparation of sulfuric acid on activated carbons (H_2SO_4/C) to make microwave absorption solid acid catalysts was recommended to further enhance their effectiveness. Due to adsorption of the coloured secondary degradation materials on the activated carbons, clear saccharified solution given by one pot MW irradiation could be directly used for further fermentation to biofuels such as ethanol.

Acknowledgement

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